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Inhibition of ATPase Activities of Brain and Liver Homogenates by Triethyltin (TET)

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by

Karen Stine Jacobs
John J. Lemasters
University of North Carolina at Chapel Hill
Chapel Hill, NC 27514

Lawrence W. Reiter
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

EPA Project Officer
Lawrence W. Reiter

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HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
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16. ABSTRACT

In vitro, TET is an effective inhibitor of both mitochondrial and Na^+/K^+-ATPase activities in subcellular fractions from brain (1,2) and liver (3). Although TET binds with high affinity to mitochondrial ATPase (4,5), it also binds to other cellular constituents including hemoglobin (6) and myelin (7). Thus, whether or not ATPase inhibition is a factor in the in vivo toxicity of TET may depend on the ability of TET to bind to ATPases in the presence of other cellular constituents. The purpose of this study was to assess inhibition of ATPase activities in liver and brain homogenates in vivo or in vitro exposure to TET.

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INHIBITION OF ATPASE ACTIVITIES OF BRAIN AND LIVER HOMOGENATES BY TRIETHYLTIN (TET)

KAREN STINE JACOBS[1], JOHN J. LEMASTERS[2], AND LAWRENCE W. REITER[1,3]
[1]Curriculum in Toxicology, and [2]Laboratories for Cell Biology, Department of Anatomy, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514 and [3]Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711

INTRODUCTION

In vitro, TET is an effective inhibitor of both mitochondrial and Na^+/K^+-ATPase activities in subcellular fractions from brain (1,2) and liver (3). Although TET binds with high affinity to mitochondrial ATPase (4,5), it also binds to other cellular constituents including hemoglobin (6) and myelin (7). Thus, whether or not ATPase inhibition is a factor in the in vivo toxicity of TET may depend on the ability of TET to bind to ATPases in the presence of other cellular constituents. The purpose of this study was to assess inhibition of ATPase activities in liver and brain homogenates following in vivo or in vitro exposure to TET.

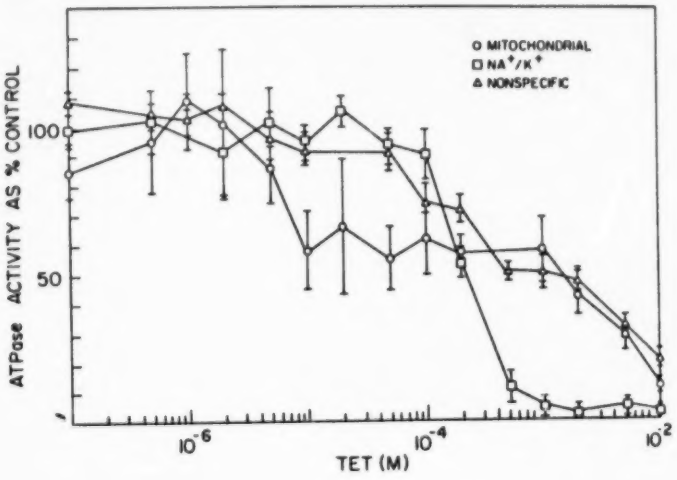
METHODS

Male Sprague-Dawley rats were injected ip with 6.0 mg/kg TET-Br or saline and sacrificed at 1 or 24 hr post-injection. Brains and livers were homogenized in 2 mM K-Hepes, 0.25 M sucrose, pH 7.4, 1-4°C. ATPase activities were determined by coupled enzyme assay (8) and by formation of inorganic phosphate (9). Results were comparable for both methods. Na^+/K^+-ATPase activity was defined as the ouabain-sensitive fraction, mitochondrial ATPase as the azide-sensitive fraction, and nonspecific activity as the ouabain and azide insensitive fraction. The Na^+/K^+-ATPase activity in liver was below detectable limits. Tissue tin levels were determined by atomic absorption spectroscopy (10).

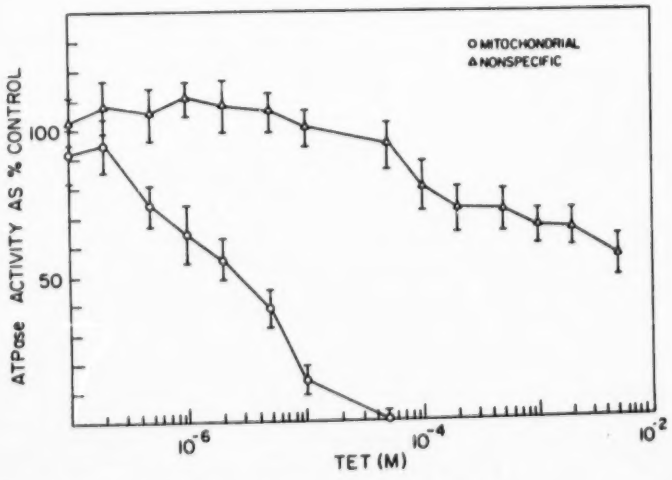
RESULTS AND DISCUSSION

The relative sensitivities to inhibition by TET for homogenate ATPases were: liver mitochondrial ATPase >> brain mitochondrial ATPase, brain Na^+/K^+-ATPase >> brain and liver nonspecific ATPase (Fig. 1 and 2; Table I). The K_i for brain Na^+/K^+-ATPase was comparable with literature values for brain microsomal fractions, while the K_i for the brain mitochondrial ATPase was much higher than for isolated brain mitochondria (1). The higher apparent K_i value for brain homogenate mitochondrial ATPase may be due to protective partitioning of TET into myelin and other lipid-rich structures, or to a basic difference in the sensitivity of

ieural and hepatic mitochondrial ATPases to TET. The latter, however, seems unikely since Moore and Brody (11) report that TET has comparable effects on neural and hepatic mitochondria.



ig. 1. Inhibition of brain homogenate ATPases by TET.



ig. 2. Inhibition of liver homogenate ATPases by TET.

TABLE I

APPARENT K_i VALUES FOR TET INHIBITION OF ATPases IN HOMOGENATES

Tissue	Na^+/K^+-ATPase	Mitochondrial ATPase	Nonspecific ATPase
Brain	2.5×10^{-6} M	2.6×10^{-4} M	1.1×10^{-3} M
Liver	--------	2.3×10^{-6} M	6.2×10^{-3} M

In order to determine if tissue concentrations of TET become sufficient to inhibit ATPases following a neurotoxic dosage, tin levels were determined in brains and livers from rats given 6.0 mg/kg TET-Br (10). Peak brain tin concentration was 17 μM at 24 hr, a concentration which was insufficient to inhibit ATPases in brain homogenates. In liver, however, peak concentration was 135 μM, which should be sufficient to inhibit hepatic mitochondrial ATPase.

To test whether inhibition of neural or hepatic ATPases occurs following a neurotoxic dose of TET, animals were treated with 6.0 mg/kg TET-Br, and ATPase activities of brain and liver homogenates measured (Fig. 3). Animals were tested at 1 hr (to evaluate early effects) and at 24 hours (time of peak tin accumulation in brain). Neither brain nor liver ATPase activities differed from controls at either 1 or 24 hr post-exposure. Although these results were predicted for brain, the

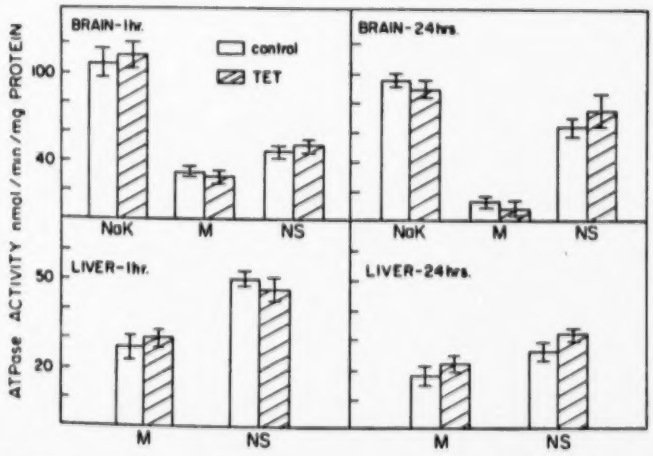


Fig. 3. ATPase activities in tissue homogenates from rats injected with TET.

expected inhibition in liver was not observed, even taking into account the dilution of the samples inherent in the ATPase assay. The lack of inhibition of liver mitochondrial ATPase after in vivo exposure to TET suggests that measured tin, even at one hr post-injection, was no longer predominately in the form of TET. TET and other alkyltins have been shown to be dealkylated in the liver by a cytochrome P-450 dependent process (12), and the metabolites may be less potent inhibitors.

CONCLUSIONS

Higher concentrations of TET are necessary to inhibit mitochondrial ATPase in brain homogenates than in isolated brain mitochondria or in liver homogenates. The greater apparent K_i for mitochondrial ATPase in brain homogenate may be due to binding of TET to other tissue constituents such as myelin. Tin concentrations in brain following a neurotoxic exposure to TET are insufficient to inhibit any brain ATPase activity measured. In liver, by contrast, tin concentrations ought to be sufficient to produce inhibition, but liver homogenates from TET-treated animals showed no difference in activity. The lack of apparent inhibition in the liver indicates that TET in liver may be bound, metabolized or otherwise rendered incapable of ATPase inhibition.

ACKNOWLEDGEMENTS

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DECREASED DENSITY OF BETA-RECEPTORS IN BRAIN CAPILLARIES OF LEAD EXPOSED RATS

STEFANO GOVONI, LAURA LUCCHI, SANDRA MAGNONI, PIER FRANCO SPANO AND MARCO TRABUCCHI

Dept. of Pharmacology and Pharmacognosy, University of Milan and Dept. of Pharmacology and Therapeutics, University of Brescia (Italy).

INTRODUCTION

An acute encephalopathy similar to that encountered in lead poisoned children can be produced in rats by feeding lead to the nursing mothers (1). On either conditions interstitial oedema and hemorrhage are found mainly at cerebellar level (2,3). Goldstein et al . (2) have shown that in the exposed rats the appearance of neurologic symptoms of lead encephalopathy is preceeded by a prehemorrhagic stage characterized by an increase of water and sodium in the cerebellum. This observation probably reflects a disturbance of cerebellar capillary permeability. Additional evidence present in the literature suggests that brain capillary function may be especially affected by lead intoxication (3-6). The function of brain capillaries is probably regulated through specific receptors for neurotransmitters present on cerebral microvessels (7). In particular, recent evidence indicates that beta-adrenergic receptors in microvessels participate to the regulation of cerebral blood flow and Blood Brain Barrier permeability (8,9). In this light we studied the effect of lead exposure on beta-receptors in rat brain microvessels as a possible mechanism inducing the reported permeability alterations.

MATERIALS AND METHODS

The rats used in our study were timed pregnant Sprague Dawley rats (Charles River, Calco, Italy). Animals were housed at constant temperature (22 °C) and humidity (50 %) and had free access to food (Nossan diet TR-M, no detectable Pb) and water (tap water; <0.01 ppm Pb). At day 16 of pregnancy mothers were caged individually and given a solution of 2.5 mg/ml lead acetate (1366 ppm Pb) or sodium acetate as control in their drinking water. Twenty mothers were used for each treatment. Litters were normalized to 10 animals 48 hours after

birth; upon weaning the offspring were separated by sex. They continued on the same drinking solution which had been supplied to their mothers. Lead treated animals showed no difference in growth rate with respect to controls. At 8 weeks of age rats were killed. Their blood lead level was 78±3 ug/dl; lead concentrations were determined by atomic absorption spectrophotometry after wet acid digestion (10). Brains were rapidly removed and microvessels were isolated from the cerebral and cerebellar cortices and used for binding studies according to Kobayashi et al.(11). In a tipical experiment six different concentrations of labelled ligand were used. For each concentration triplicate samples were run for total and non specific binding. Pooled microvessels isolated from the cerebral cortices of 8 rats or from the cerebellar cortices of 16 rats were needed for a single experiment.

^{125}I-iodocyanopindolol (2 Ci/umol.; Amersham Radiochemical Centre, England) was used as labelled ligand. Non specific binding was measured in the presence of 20 uM unlabelled isoproterenol. Protein content was measured according to Lowry et al. (11).

RESULTS

Figure 1b) shows that the number of beta receptors is decreased by 29 % in the microvessels purified from cerebellar cortices of lead exposed rats, while the affinity is unmodified (B_{max}: 95±9 f.moles/mg protein, K_d: 58±5 pM and B_{max}: 68±6* f.moles/mg protein, K_d: 59±6 pM for controls and lead treated rats respectively; values are means ± S.D. of four independent experiments; *P <0.01).

On the contrary, no changes were observed in the beta-receptor number or affinity of microvessels isolated from the cerebral cortices of intoxicated rats respect to controls (Fig. 1a; B_{max}: 111±8 f.moles/mg protein, K_d: 69±7 pM and B_{max}:116±10 f.moles/mg protein, K_d 72±8 pM for controls and lead treated rats respectively; values are means ± S.D. of four independent experiments).

DISCUSSION

Various experimental observations suggest that adrenergic neurons in the Locus

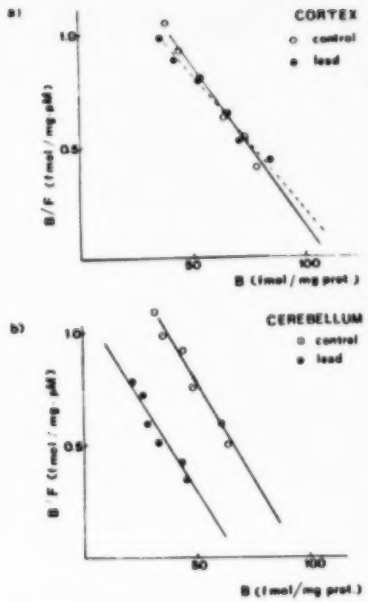

Fig.1 Scatchard analysis of (^{125}I)-Iodocyanopindolol specific binding microvessels isolated from cerebral (a) and cerebellar (b) cortices of cont and lead treated rats.
The figure shows a representative experiment out of four.

Coeruleus innervate cerebral microvasculature (13,14). The hypothesis o neuronal control of brain microcirculation is further supported by identification in cerebral capillaries of alpha- and beta-adrenergic recep (11,15). Lead exposure is known to induce changes in central and periph catecholamine metabolism (for a review see 16), suggesting that lead interfere with basic neurochemical processes. In this line, the decreas the number of capillary beta-receptors observed in the present study either reflect an impairment of the adrenergic regulatory mechanism cerebral microcirculation or a direct toxic effect of circulating lea these receptors.

It has been postulated that an altered permeability of cerebe capillaries is the earliest functional defect in the pathogenesis of

encephalopathy (6). On this line the decrease of beta-receptor density in microvessels prepared from cerebellar cortices of lead exposed rats may have a functional importance in determining an altered capillary permeability during lead intoxication. Notably, with the dose of lead used in our protocol, no changes were observed in the number of beta-receptors of cerebral cortex microvessels. This observation is consistent with previous ones (6,7) suggesting that capillaries of the cerebellum may be more susceptible to lead toxic action than capillaries of other brain regions.

The present study raises the possibility that lead poisoning may be accompanied by changes of brain capillary receptor density which may be an early biochemical marker of lead toxic actions on cerebral vascular function.

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